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ON THE INFLUENCE OF TEMPERATURE ON THE EXCRETION OF THE HIBERNATING FROG, RANA VIRESCENS KALM.

H. C. VAN DER HEYDE.[1]

From the department of physiology and physiological chemistry of the West Virginia University Medical School, Morgantown, W. Va.

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Almost all vital phenomena have been studied as to the influence of temperature on their magnitude. In his excellent book on "Temperatur und Lebensvorgänge," [2] Kanitz gives a summary of the work done in this field, and tries to show that the R-G-T rule of van't Hoff-Cohen holds true also for the reactions of organized material. In Loeb's laboratory it was found by Snyder and Robertson that the heart-beat of invertebrates and of the lower vertebrate follow this rule. These observations have been confirmed and amplified by several authors. For the mammalian heart also some investigators—among them Snyder—tried to show that its frequency follows van't Hoff's rule. Other observers however could not agree on this point and emphasized the complexity of the phenomenon, which would make it improbable that this rule, which holds true for simple chemical reactions, could be applied to these more complex phenomena. The rhythm of breathing, the velocity of conduction in the nerve, the activity of the muscles, the geotropic and phototropic movements of plants, the effect of poisons, the length of life, the rate of development and growth, all have been studied from this point of view, and a great diversity of opinions exists as to whether the rule of van't Hoff holds true or not.

[1] Thanks are due to Mr. R. Goldberg, who made some of the total nitrogen, urea and ammonia determinations for me.

[2] Aristides Kanitz, "Temperatur und Lebensvorgänge," *Berlin. Gebr. Borntraeger*, 1915.

The advocates of the confirming answer (as for instance Snyder and Kanitz) explain the little differences between the theoretical values and the experimental results as due to errors in our methods, while those who deny the parallelism between the reactions of organic material and those of ordinary chemistry emphasize these differences and sometimes claim a regular decrease in the value of the temperature coefficient, as Krogh and Ege did in a controversy with Snyder.

Several authors studied the influence of temperature on metabolic processes. The CO_2 assimilation of plants was shown by Matthaei and numerous other authors to follow the rule of van't Hoff. The frog's metabolism was first studied by Hugo Schulz.[3] The principal importance of his work was that he showed that temperature has a tremendous influence on the frog's metabolism. The frog's output of CO_2 is according to him more than 16 times as much at 25° as at 0°. Aubert[4] gave more accurate figures, but they can still not yet be used for checking them up with the formula of van't Hoff. Vernon's publications,[5] in which he tried to show that between 2° and 17° the CO_2 output was constant, provoked some other papers; the results seem to be due to the sudden changes in temperature to which he subjected the animals. The same fact, a very slow increase in metabolism between 10° and 20°, has also been found in *Cyclodus gigas*, a lizard, by C. J. Martin[6] and in the work of some other investigators.

Experiments.

In my own experiments I tried to determine the influence of temperature on the excretion of winter frogs. Ten animals were kept for 24 hours in a small aquarium. The bottom was covered

[3] Hugo Schulz, "Ueber das Abhängigkeitsverhältnis zwischen Stoffwechsel und Körpertemperatur bei den Amphibien," *Pflüger's Arch.*, 14, 78–91, 1877.

[4] Hermann Aubert, "Ueber den Einflusz der Temperatur auf die Kohlensäure Ausscheidung und Lebensfähigkeit der Frösche in sauerstoffloser Luft," *Pflüger's Arch.*, 26, 293–323, 1881.

[5] H. M. Vernon, "The Relation of the Respiratory Exchange of Cold-blooded Animals to Temperature," *Journ of Physiol.*, 17, 277–292, 1895. H. M. Vernon, "The Relation, etc., Part II," *Journ. of Physiol.*, 21, 442–496, 1897.

[6] C. J. Martin, "Thermal Adjustment and Respiratory Exchange on Monotremes and Marsupials," *Transact. Roy. Soc. London*, (B), 195, 1–37, 1902.

with some distilled water; care was taken to keep this quantity as constant as possible for reasons given in a previous paper.[7] By taking 10 animals at the same time the individual differences were eliminated as much as possible. The aquarium was placed in a larger water container in which the temperature of the water could be automatically regulated, whereas a stirrer moved by a motor kept the water constantly in motion. After 24 hours the urine was centrifuged—to remove the skin particles and the fæces—and then measured. In this urine I ran total nitrogen, urea, ammonia and uric acid determinations.[8] A difference of ten degrees was chosen because the classical formula of van't Hoff's rule speaks of 10°. Higher temperatures than 31° could not be used because a temperature of about 33° is fatal for the frog as appeared in some experiments in which I found all animals dead after having them kept for some time at higher temperatures. Even in the 31° experiments some of the frogs were very faint and near death after 24 hours.[9] This is probably the reason why my figures for this temperature were much more irregular than the rest—in one of the experiments I got for instance 70 mgm. total nitrogen. The results are given in Table I.

TABLE I.

Temperature.	Total Nitrogen.	Urea and Ammonia Nitrogen.	Urea.	Ammonia Nitrogen.	Ammonia.	Uric Acid.	Nitrogen in it.
1°	6.55	6.5	10.5	1.6	1.9	0.0	0.0
11°	10.45	9.2	13.8	2.8	3.4	0.13	0.04
21°	16.3	14.6	23.9	3.5	4.2	0.30	0.10
31°	59.03	50.8	60.4	22.7	27.6	0.83	0.28

[7] H. C. van der Heyde, "Studies in Organic Regulation, I., The Excretion and the Blood-Picture of the Hibernating Frog," *Journ. Biol. Chem.*, XLVI., 1921, p. 421.

[8] The total nitrogen, urea and ammonia figures are the average of three series of determinations on each of which the determinations were made *in duplo*. It should be noted that the figures of one series were not identical with those of the others; but that though the way of increase of each series was identical the absolute values showed some variation. The uric acid figures have only been determined in one series. As previously I wish to state that my trust in the uric acid figures is not very great for reasons given in my previous paper (7).

[9] It seems that the highest temperature which *Rana pipiens* tolerates according to the experiments of Cameron and Brownlee (*Transact. of the Royal Soc. of Canada*, Ser. III., Vol. IX., p. 67) is even lower.

The figures for the total nitrogen are graphically represented in Fig. 1.

It is clear that temperature has in reality a tremendous influence on the frog's catabolism. From 0° till about 20° this increase is only relatively slight. After 20° however the curve rises almost vertically. When we compare our curve with that

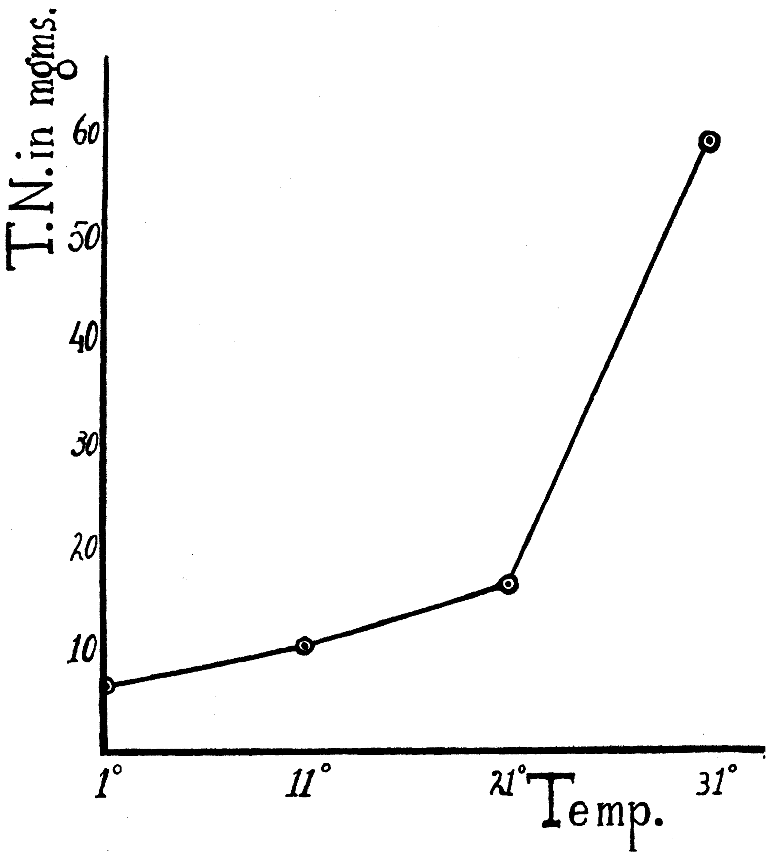

FIG. 1. The influence of temperature on the total nitrogen excretion.

given by Lusk[10] after the figures of H. Schultze, we notice a striking similarity between the two curves. It is clear moreover that this fact has a great biological importance. The frog in his hibernating stage at 4° in the mud can not have the intensive metabolism which the frog in midsummer "as he sits on the

[10] Graham Lusk, "The Elements of the Science of Nutrition," 3d edition, W. B. Saunders Co., Philadelphia and London, p. 115.

river bank and snaps at passing flies" sustains. The change in temperature of its blood causes its tissues to show a much more intensive metabolism. Krogh in his numerous studies on the influence of temperature on poikilothermous animals gave as his opinion that the influence of temperature on the animal's metabolism is of double nature. On the one hand the basal metabolism is increased, on the other hand the tonus of the muscle is increased and causes in that way an increased muscle metabolism. The latter process is regulated by the central nervous system and in fact he observed that in decerebrated animals the temperature did not have as much influence on the CO_2 output as in normal animals. Moreover Krogh is of the opinion that a regular decrease can be observed in the temperature coefficient. For this reason I figured out the temperature coefficients of my own experiments which are given in Table II.

TABLE II.

Range.	Coeff.
1–11°	1.6
11–21°	1.56
21–31°	3.6

Instead of a decrease we notice an increase. We must however keep in mind that these experiments have been made on normal animals. The quoted experiments of H. Schultze give the same result.

I do not dare to say in how far the rule of van't Hoff holds true for this case. I believe that we can not be careful enough in drawing conclusions on this point. The reactions of the organism as a whole can not but with extreme care be compared with simple chemical reactions. Not only the two factors emphasized by Krogh play a rôle in the processes of which we see the final result in our urinary analysis, but also the blood pressure, the water intake which has been shown by Overton[11] to be very strongly influenced by temperature, and the function of the kidneys. For this reason it seems not very probable that the final result of all these processes should be comparable to a

[11] E. Overton, "Neunundreiszig Thesen über die Wasserökonomie und die osmotischen Eigenschaften der Amphibienhaut," *Vorl. Mitt. physik. medizin. Gesellsch.*, Würzburg, N. F., 36, 282, 1904.

simple chemical reaction. When however we calculate the temperature coefficients we see that they are not constant enough to give us the right to the conclusion that van't Hoff's rule holds true in this case, but on the other hand they do not prove the contrary.

To one remarkable phenomenon which I observed in my experiments I might still draw attention. As stated in my

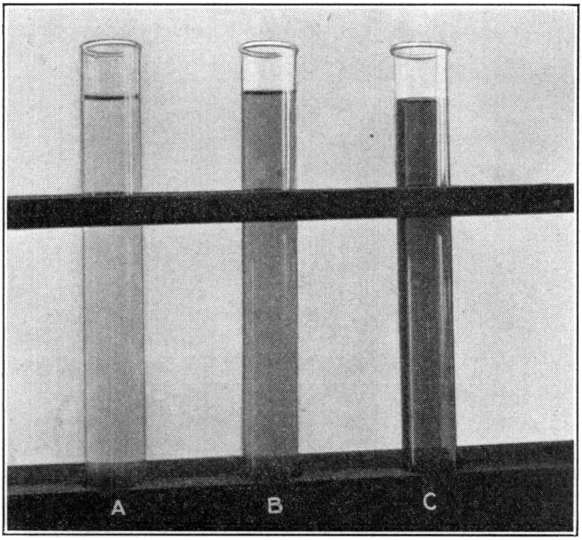

FIG. 2. Influence of temperature on the appearance of the yellow pigment of the frog's urine. *A*, 11°; *B*, 21°; *C*, 31°. As the color of the 1° urine did not differ from that of 11° only these three have been photographed.

previous paper pure frog urine as obtained from frogs in the vivarium in which the average temperature is below 10° is absolutely waterclear. Toda and Taguchi however in their paper on the inorganic constituents of the frog's urine observed a slight yellow color. Their experiments were made on summer frogs. Now it is very remarkable that indeed this pigment appeared in my experiments in which higher temperatures were used. Fig. 2 shows a picture of three samples obtained in three different experiments at 11°, 21° and 31°. The increase in color which was even more striking than this picture shows runs completely parallel with the nitrogen content as represented in Table I. and Fig. 1.

ZUSAMMENFASSUNG.

Die Temperaturabhängigkeit der Stickstoffelimination von überwinternden Frösche (*R. virescens*) wurde studiert. Die Zahlen sind in Fig. 1 graphisch dargestellt. Ein gelbes Pigment tritt auf wenn höhere Temperaturen benutzt werden, die Intensität der Färbung geht dem Stickstoffgehalt und der Temperatur parallel (Fig. 2).